" AUGMENTATIVE ROLE OF PLANT GROWTH PROMOTING BACTERIA (PGPB) IN MODULATING RESPONSES AGAINST MITIGATION OF SALT STRESS IN *TRIGONELLA FOENUM-GRAECUM*"

A DISSERTATION

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Submitted by:

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CANDIDATE'S DECLARATION

I hereby certify that the work which I presented in the Major Project-II entitles "**Augmentative role of plant growth promoting bacteria in modulating responses against mitigation of salt stress in *Trigonella foenum-graecum***" in fulfilment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own, carried out during a period from Jan-April, under the supervision of Prof. Jai Gopal Sharma. The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University. The work has been communicated in Scopus indexed journal with the following details:

Title of this paper: **Augmentative role of *Piriformospora indica* fungus and plant growth promoting bacteria in modulating responses against mitigation of salt stress in *Trigonella foenum-graecum***
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SUPERVISOR CERTIFICATE

To the best of my knowledge, the above work "**Augmentative role of *P.indica* fungus plant growth promoting bacteria in modulating responses against mitigation of salt stress in *Trigonella foenum-graecum***" has not been submitted in part or full for any Degree or Diploma to this university or elsewhere. I, further certify that the publication and indexing information given by the students is correct.

Place: Delhi

PROF.JAI GOPAL SHARMA

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Date: June 1, 2021

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CERTIFICATE

This is to certify that the M.sc dissertation entitled "**Augmentative role of plant growth promoting bacteria in modulating responses against mitigation of salt stress in *Trigonella foenum-graecum***" in partial fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 7-Jan-2021 to 28-May-2021. The information and data enclosed in this dissertation is original and has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 28 May, 2021

PROF. JAI GOPAL SHARMA
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(SHATRUPA SINGH)

ABSTRACT

An experiment was conducted to evaluate the role of plant growth promoting bacteria (PGPB) in mitigating salinity stress in *Trigonella foenum graecum*. Plants were subjected to three different levels of salinity viz 0, 70 and 150 mM NaCl (electrical conductivity value 0.01, 7.67 and 15.50 mS cm^{-1}, respectively) using a completely randomized design experiment. PGPB showed positive effects in mitigation of salinity stress in fenugreek plants and elevated various growth responses viz. shoot and root length, shoot and root dry weight, leaf area and number of leaves as compared to uninoculated plants. Microbial inoculation significantly enhanced the physiological responses viz. photosynthetic rate, stomatal conductance, transpiration and internal CO_2 as compared to uninoculated plants. Biochemical aspects like carotenoids, chlorophylls, nitrogen and protein content were also increased in the microbial inoculated plants as compared to uninoculated plants. PGPB was very effective than in mitigating salinity stress in fenugreek plant. The findings of this study revealed that PGPB inoculation can help the plants to overcome the deleterious effects of salinity stress in fenugreek plants.

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CHAPTER 1 INTRODUCTION

1.1 Salinity stress

A crucial challenge for world agriculture is meeting the rising global population's food demand which is currently growing at about 1.05 percent per year (World Population Prospects, 2019). Several biotic and abiotic stresses have a significant impact on plant growth, productivity, yield and food quality. Damages or diseases caused by a variety of pests or pathogens are referred to as biotic stresses whereas salinity, rising temperatures, declining freshwater supplies, heavy metals and other chemical pollutants are example of abiotic stresses which necessitate an integrated solution, collective intervention, and extensive research in order to resolve and adapt (Jogawat et al., 2013).

Soil salinity is the most harmful among all the abiotic stresses (Daliakopoulos et al., 2016). Salinization is the existence of various types pf salt ions in soil since plants have a very diverse reaction to soil salinity, and while growing in salinity conditions these factors completely influence their productivity (Shilev 2020).

Based on the origin of the salinity, it classified as primary and secondary. All of these primary salinity sources may be found on land and occur naturally: salinity is gained through weathering of rocks, salinity from shallow brackish groundwater, salinity in saltwater seeps into the coastal region, and restricted drainage brings more salty water to the shore. Secondary salinization is a result of human activity; it can develop when drainage is inadequate, industrial wastewater is being dumped, excessive use of fertilizers, the loss of natural plant cover, and flooding with saline water (Wang et al. 2003). In protected cultivation, it grows exceedingly. Furthermore, it has affect on irrigated fields which are having water shortages, high temperature, and evapotranspiration

1.2 Status of salinity Worldwide

Salinization of agricultural land happen mainly because of the deposition of salt in soil (Bharti et al., 2016).Salinity affects over 20% of agricultural land worldwide and the problem is only getting worse (Gupta and Huang., 2014). About half of the cultivated land would be salinity affected by 2050 as per the estimations. An estimate number of 6.7 MHA of area in India is also effected by salinization with Gujarat having the largest volume of almost 71 percent of the overall salty soils in India. The most influenced region due to salinity internationally include the Asia Pacific and Australia. In America, there are 12223.41 million

ha cultivated land of which 130.5 million are saline whereas in Europe 17.30 percent land part is affected (FAO, 2019; World Population Prospects, 2019); in Africa, 6.40% of total agricultural area is salinity affected (FAO, 2015). (Kumar et al., 2020)

1.3 Effect of salinization on plant growth and productivity

Photosynthesis is affected by salt stress in both long and short terms respectively. Salt stress which is of short term is very quick and happens within a span of time causing limitations in stomata restrict photosynthesis caused by salt resulting in a reduction in uptake. of carbon whereas the Long-term affect is that the salt gather in the young leaves and affects photosynthesis, this reduces the amount of chlorophyll and carotenoids and cause changes as increase in the chlorophyllase activity may cause a decrease in chlorophyll content (Saravanavel et al. 2011). Salinity stress can be seen to effect stomata size and also its density, which leads to depletion in the conductance of the stomata. The thylakoid of chloroplast forms also become disordered as they are exposed to salt and the number and size of plastoglobuli increases as well. The Plants which are exposed to elevated salt concentrations are shorter and have smaller leaves with pale colour (Shilev 2020). It is also observed that salt stress affects the shoot and the reproductive development of the plant. Plant growth its flowering, seed germination stomatal closure, and cell death is regulated by Nitric oxide. The presence of Cl salts in the external medium causes decreased nitrate reductase activity under salt tension which causes reduced nodulation, leghaemoglobin content, and nitrogenase activity. Horticultural crops (spinach, potatoes, tomatoes, lettuce) and cereals (maize, wheat, rice, legumes) are sensitive to salinity stress which reduces the yield up to 50–75% (Shilev 2020)A plant that is under the influence of salt stress goes through series of morphological, physiological and molecular modifications, eventually obstructing its maturation

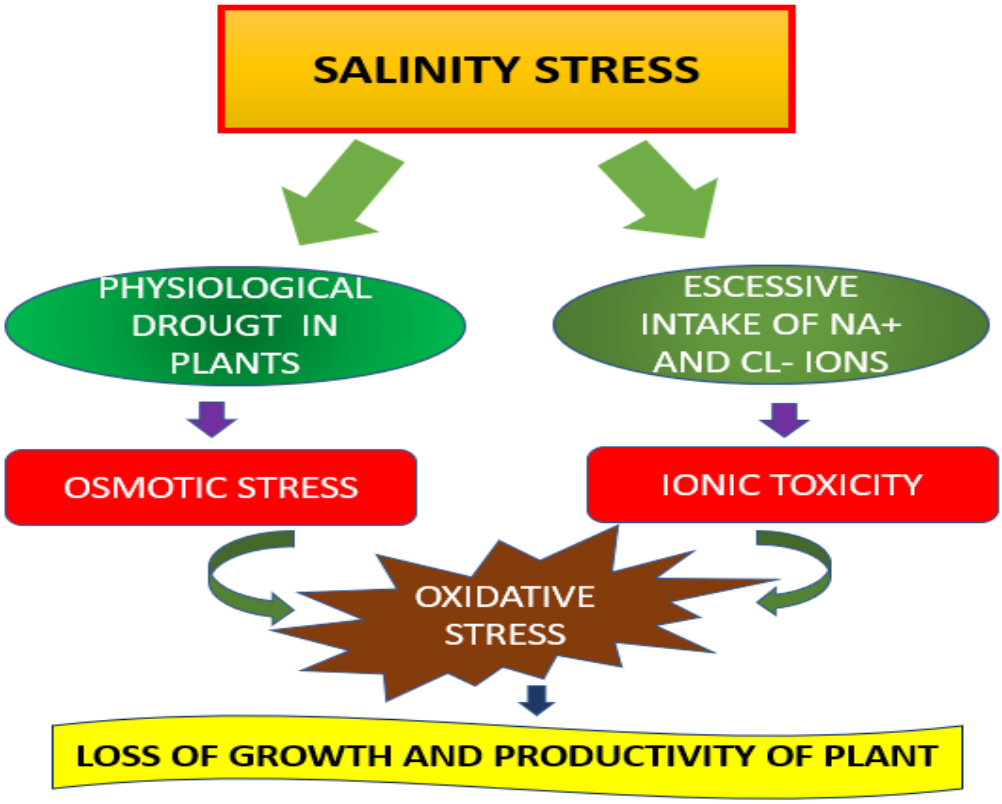


Figure 1- Effect of Salinity on Plants

There were several reports that suggest salinity raises Na+ levels, which results in Calcium and Pottasium loss. The water balance and ion homeostasis are upset because to the accumulation of poisonous sodium and chloride ions; this subsequently influences metabolic processes, hormonal state, and enzyme, photosynthesis, transpiration, and nutrient translocation . Severely restricting the quantity of salts in the soil can make it difficult for plants to absorb key nutrients that are required for growth of plants. Competitive interactions arise between ions with hazardous potential and ions with nutrition potential for protein transportation in the root cells, after which it has a large impact on the movement of nutrients and distribution (Tester and Davenport 2003). The plant's nutritional content will be lowered as a result of this contact. Plant viability is also threatened by oxidative stress due to the presence of Reactive oxygen species. (Turan et al. 2012). Additionally, ROS are able destroy plant membranes, eventually resulting in DNA damage (Selvakumar et al. 2012).. Salt stress effects premature mortality through increased production of ethylene and abscisic acid. As a result, leaf and petal drop (abscission) and tissue ageing (senescence) are accelerated, as well as plant growth retardation. Ensuring the mutually beneficial symbiosis starts with bacteria’s movement to plants root, then colonization by root hairs, cortex division, and root hair

deformity all happening quickly, starts with successful symbiosis establishment (Van Rhijn and Vanderleyden 1995).

Salinity disturbs the beneficial bacteria; symbiotic relationship with plants. Bacterial attachment (adsorption and anchoring) as well as nodulation and nitrogen fixation are altered when protein molecules are involved in the earliest phases of attachment (adsorption and anchoring) are affected, both of which lead to disturbances in symbiotic relations (Nabti et al.2015).

1.4 Techniques to prevent crop loss due to salinity

to manage salinity stress in agricultural crops Salinity is a global limiting factor for agricultural yield, as mentioned above. There is a serious difficulty in the creation of effective, cheap, and flexible ways for handling environmental stress. Saline soils can be treated in several ways. One approach is to use physical, chemical, or biological means. Washing, leaching, and scraping (mechanical removal of the salt layer of the topsoil, and the bottom layer is utilized cultivation) are some of the treatment methods; however, this method has had limited results and is expensive. (Qadir et al. 2000). Using chemical amendments puts our ecosystem at risk and also makes crops more vulnerable to illness, since certain helpful bacteria go extinct. Moderately low stress can be alleviated with non-genetically modified and genetically supple plants or agricultural practices that increase. These methods are time-consuming and thus economically unsustainable.

1.5 Microorganisms in mitigating salinity stress

Microbes are less expensive and have tremendous stress-relieving capacity Plant microbial association boosts the plant growth and production under salt stress (Enebe and Babalola). Since saline ecosystems have insufficient nitrogen, nitrogen input is needed in these conditions (Wang et al.2018). The role of *Bacillus amyloliquefaciens* has been confirmed to improve chlorophyll content in maize seedling under salinity stress (Chen at al 2016). *Massilia sp*. and co-culture of *Rhizophagus intradices* have improved the nitrogen in maize shoots dramatically (Krishnamoorthy et al.2016). *Pseudomonas putida* inoculation in soyabean plant increased the shoot length, chlorophyll content, biomass (Kang et al.). Under high salt conditions, the photosynthetic pigment content was substantially increased in *P. indica* inoculated rice seedlings. *Brachybacterium saurashtrense, Brevibacterium casei* and *Haerero halobacter* increased total biomass in *Arachis hypogea (*Shukla et al.2012).

Salt tolerance has been identified in host plants provided by PGPB which helped the plants' ability to survive in adverse situations There is conclusive evidence for the efficacy of salt stress alleviating plant growth-promoting rhizobacteria (PGPB) based on many biological activities.

1.6 Plant growth Promoting Bacteria (PGPB)

Plant growth-promoting bacteria (PGPB) are quite interesting to researchers, particularly in the agricultural world, since they aid in the management of crop health in the rhizosphere as well as the enhancement of crop development, yield, and overall fitness. (Dee Salmon et al. 2005). Kloepper and Schroth (1978) originally defined crop development supporting rhizobacteria as microbes which populate over crop roots after upon seed inoculation and improve crop production.

1.7 PGPB and salt tolerance

There are several possible applications for PGPB in agriculture, all of which are respectful of the environment. Knowing more about these bacteria might help agricultural soils to decrease any use of fertiliser inputs and also save money. Such microbes enhance development of seed, roots, shoots, & leaves. These characteristics contribute to increasing the number of seeds, the rate of root development, and the thickness of the root and stem. Higher leaf area also contributes to higher yields. The chlorophyll level also goes up. In addition, P & N uptake increases. Different rhizospheric bacterium contribute to crop survival and growth in a number of ways, but these bacteria penetrate roots by exuding nutritional substrates (Vaacheron et al. 2013). When it comes to plant-microbe interaction, root exudates secreted by plant have a role to play (Ilangumaran and Smith 2017). Bacteria in the gut get nutrients via these compounds, and they function as chemical signals that plants use to interact with their microbiome (Badri etal. 2009). Non-biotic factors control was shown to be altered by PGPR treatment through multiple mechanisms that result in system’s endurance.(Yaang et al., 2009). Various PGPRs were studied for potential ability to enhamce plants & water interactions, ion homeostasis, and photosynthesis proficiency in salt-stressed vegetation, although their amelioration processes are complex and frequently unknown.

1.8 Contribution of PGPB in elevating plant salt stress

Because of the many research investigations on variety of plants, current investigations have shown that various bacterial species have been discovered to be PGPRs. The range of PGPR diversity is rather wide, based on the kind of species, soil, and nutrient accessibility. The bacterium Pseudomonas and Bacillus are found in several locations and are the most well investigated members of the category of PGPR. Also, plants are resistant to salt stress because of their exopolysaccharides, which provide them with protection from desiccation and ion toxicity (Arora et al. 2012). protection from numerous abiotic stressors including as drought, chilling damage, salinity, metal toxicity, and extreme temperature stress (Sandhya et al. 2009; Timmusk and Wagner 1999). (Ali et al. 2009, 2011). In this experiment we analyzed the beneficial role of PGPB association in fenugreek plants during salinity stress.

CHAPTER 2 LITERATURE REVIEW

Due to the reduced salt stress impact, the plant growth promoting potentials of PGPB seem to be considerable. Since PGPB has the capability to counteract salt stress, the organisation has the ability to have a huge impact. creative Data given in Table 14.1 (continued) Using the PGR Crops species, you get a big benefit. Hyperlinks Lipoferrin-encoding bacterium Brassica oleracea" Canola plant development may be optimised reduce the antioxidant enzyme's level Increase the amount of microelements. Aspergillus tumefaciens The pseudomonas strain known as Arachis hypogaea Boost the development of the peanut plants Reduce the quantity of reactive oxygen species (ROS).This Pseudomonas putida strain R4 the R5 strain of Pseudomonas chlororaphis Torenia gossypina Increase the ability of seeds to germinate and seedlings to grow Egamberdieva and colleagues (2015) variegated Japanese bellflower B. thuringiensis Nebe17 Common bean changes in stress response proteins, including increased protein PEP carboxylase upregulation. Pseudomonas strains are able to promote crop development under salt stress, as shown by Egamberdieva and Kucharova (2009). Salinity had a detrimental influence plant length and biomass of the soybean crop, although the effects were ameliorated by using PGPR. Pseudomonas pseudoalcaligenes and Bacillus pumilus were used to promote the germination of rice seeds under salinity. The seed germination of tomato was raised, and to a greater extent, seedling dry weight was improved up to 120 mM of sodium chloride. This study showed that adding Pseudomonas to cowpea plants cultivated under salt stress led to a rise in the carotenoid levels. As a result of PGPB strains Pseudomonas putida R4 and Pseudomonas chlororaphis R5, cotton rhizosphere salinity is relieved by IAA phytohormone synthesis. Paulucci have discovered a novel bacterium, Ochrobactrum intermedium, which produces indoleacetic acid, siderophores, and deaminase enzyme in response to temperature and salinity, making it better for the development of peanut plants under salt stress.Curtobacterium flaccumfaciens, was identified to provide tolerance to *Hordeum vulgare* plants from salinization. It was also observed to boost germination and provide salt tolerance in the plant (Cardinale et al. 2015). Systemic resistance induction was also observed following application of Serratia marcescens, which can lower wheat resistance to both biotic and abiotic stress by increasing the concentration of different osmoprotectants.

CHAPTER 3 METHODOLOGIES

3.1 Plant material

Trigonella foenum-graecum L. seeds obtained from National Seeds Corporation, Pusa, New Delhi. The classification and description of the plant is given below:

Kingdom: Plantae

Order: Leguminosae

Family: Fabaceae

Subfamily: Trifoliae

Genus: Trigonella

Species: T. foenum-graecum L

Habit

Stems of fenugreek are 20-130 cm long, straight, usually ascending, branched, occasionally simple, sparsely pubescent, generally hollow, and anthocyanin-tinged at the base or all the way up. The first leaf is simple, trifoliate, oval or orbicular in shape, with an entire border and a long petiole. Stipules are pretty big and softly haired. Leaf petiole is enlarged at the top and attenuates beyond its point where the lateral leaflets join. Petiolules are little cartilaginous. Simple, sparse hairs developed on the underside of petioles and petiolules. Leaflets are ovate-orbicular to oblong-lanceolate, 1-4 cm long, almost equal, finely hairy, and dentate toward the apex, with dentation more pronounced in top leaves than lower leaves. Flowers in leaf axils, usually twining, but occasionally single. Soft hairy calyx with teeth as long as the tube and half the length of the flower. Long pale yellow corolla with a violet tint at the bottom. Pods are curvy, sometimes straight, and have transitory hairs. They are 10-18 cm length and 3.5 x 5 cm wide. The pod is green or reddish in colour before ripening; when mature, it is light straw or brown in colour and contains 10-20 seeds.

Economic Importance

Fenugreek is one of India's most important cash crops. It's a dicotyledonous annual herb used as vegetable and forage. The seeds are used as human and animal food, and for industrial and therapeutic applications (whole, crushed, in flour, or roasted). (Petropoulos 2002). Because it is a low-water-requirement dry-land crop, fenugreek cultivation is promoted to minimize

irrigation costs, conserve water, minimize eutrophication of surface waterways, and minimise pollution of ground water sources (Basu et al. 2004). It's also a superb soil restorer and is frequently used as a cereal break crop. Fenugreek is salt tolerant and can adapt to different environmental conditions (Duke 1986; Petropoulos 2002)

Figure 2- (A) Fenugreek Seeds and (B) Potted Plant of Fenugreek

3.2 Experimental design and Growth conditions

Experiment was conducted in the horticulture, Delhi Technological University, Delhi, India. During the *Trigonella foenum-graecum* growing season (December-March), under natural

light, temperature, and humidity conditions. The 3 x 6 factorial experiment was designed for PGPR) with 2 conditions: Treated or non-treated with three Nacl concentrations (0,70, and 150Mm Nacl). Thus eighteen combinations were set up in a three-times repeated randomised full block configuration for microbe PGPB.

3.3 PGPB Treatment

For Bacterial treatment seeds were mixed with 20g of PGPB inoculum (*Azotobacter chroococcum, Enterobacter asburiae and Lactococcus lactis*) (1:1:1) for each pot at the time of sowing.

Figure 3- Experimental setup showing pots with seeds in soil uninoculated and inoculated with PGPB

3.4 Soil and salt treatments

Seeds of fenugreek were sown in each pot having 4 kg of an autoclaved soil (mixture of sand, silt and clay).To kill any existing bacteria, the Before being autoclaved for 15 minutes at 121°C and 15 pressure, the soil was filtrated and combined equally with sand. Each pot received four kg of autoclaved soil. Seeds were added to each pot, 3 cm deep and was mixed with soil to sandwich the seeds. Eight sterilized seeds were sown in each plastic pot. The soil had a pH: 7.2, organic matter:1.3%, available N: 185 mg g^{-1}, Available P: 49.4 mg g^{-1}, Available K^{+}: 295 mg g^{-1}, Mg^{2+}: 230 mg g^{-1}, Zn $^{2+}$: 6.8 mg g^{-1}, Fe^{3+}: 11.9 mg g^{-1}, Cu^{2+}: 3.99

mg g^{-1}, Mn^{2+}: 6.98 mg g^{-1}. The plants were grown in greenhouse conditions (Temperature: 23-28 °C; relative humidity: 65 ± 5% and light intensity: 1500 lux). The NaCl treatment began 30 days following plant development. 50 ml of recommended NaCl solution was added in each pot sequentially for 7 days In control 50 ml of distilled water was added in each pot till 45 days after sowing. Autoclaved tap water was used for irrigating the plants twice in a week. Plants were grown.Upon addition of NaCl solution, the electrical conductivity of soil extract were increased to 0.01, 7.67 and 15.50 mS cm^{-1} in the 0, 70 and 150 mM NaCl salinity levels, respectively. Autoclaved tap water was used for irrigating the plants twice in a week. Plants were harvested by uprooting the entire plant manually after 45 days of sowing under N60P80K40 combination of fertilizers using Urea, MOP (Muriate of Potash) and DAP (di-ammonium phosphate).

3.5 Growth parameters

After 64 days, the plants were harvested and separated into roots and shoots.To eliminate any sticking particles, the root and shoot were rinsed thoroughly in tap water and blotted dry. The number of leaves, fresh weight and lengths of root and shoot were measured immediately using a weighing balance and scale, respectively. Thereafter, the plant tissues were wrapped separately in aluminium foil and kept in oven for 72 hours at 70 - 80 °c to record the dry weights

3.6 Physiological Parameters Measurement

Physiological parameters (photosynthesis, stomatal conductance, transpiration, internal CO2 and Leaf area measurement) was performed on portable photosynthesis system Li-6400XT IRGA (Infra-red gas analyser) Department of botany, Delhi University

Figure 4.1- (A) Measurement of Physiological Parameters on IRGA (B) portable photosynthesis system Li-6400XT IRGA

3.7 Leaf Area and Number of Leaves Measurement: The numbers of leaves were counted. Leaf area measurement was performed using leaf area meter (CID Bio-sciences, CI-202 Laser area meter).

3.8 Chlorophyll and Carotenoid Estimation

Chlorophyll content was measured according to Hiscox and Israeltam (1979). Fresh leaflets (0.1 g) were cut into small pieces and put into a vial of 7 mL dimethyl sulfoxide (DMSO). The leaf tissue in the vials was incubated at 65°C until it turned white. The extracts were poured to a measuring cylinder, DMSO was used to make up the entire volume to 10 ml. The extract's absorbance was measured at 645 and 663 nm for chlorophyll content, 480 and 510 nm for carotenoid content and the concentration of chlorophyll and carotenoid was calculated using the following formulas respectively.

Chlorophyll a (mg/g fresh weight) = 12.7×D663-2.69D645 × Volume/1000× sample weight

Chlorophyll b (mg/g fresh weight) = 22.9×D645-4.68D663 × Volume/1000× Weight of sample

Total chlorophyll (mg/g fresh weight) = 20.2×D645+8.02D663 × Volume/1000× Weight of sample

Carotenoid (mg/g fresh weight) = 7.6D480-1.49D510 × Volume/1000× Weight of sample

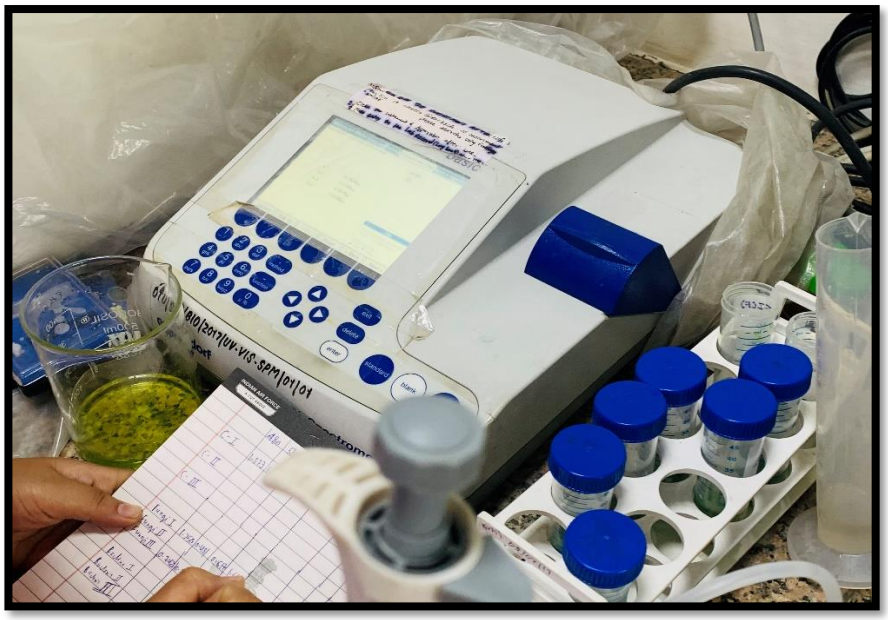


FIGURE 4.2- Measurement of Absorbance of Leaf Extracts on UV spectrophotometer for Estimation of Chlorophyll and Carotenoid content

3.9 Protein Estimation

Sample was prepared by grinding oven dried leaves using suitable laboratory grinder and sample was weighed to accuracy of 0.2g into a 250ml digestion tube. Digestion was performed on KJELDAHL digestor unit for 60 minutes with 0.2g sample,7g K2SO4, 0.8g CuSO4.12 ml concentrated H2SO4 was added. Tubes were shaken gently to wet the samples. Exhaust was positioned and scrubber was turned on. Rack was removed with exhaust and left to cool for at least 15 minutes. A reagent blank was included in the digestion (all reagents added to the digestion tube, only sample excluded). Distillation was performed automatically on KJELTEC 8200 unit.30 ml of Boric acid (receiver solution) was added to receiver flask. 80 ml H2O and 50 ml 40 % NaOH was added automatically to dilute the digest. Distillate was titrated with 0.1N HCL(Standardized) as titrant. blank was carried out earlier to each set of samples. % Nitrogen and % Protein was calculated.

% N = (T-B) ×N×**14,007×100/ weight*sample* mg**

T = Sample titration B = Blank titration N = Normality of titrant

% Protein = N×F

F = 6.25 for Fenugreek

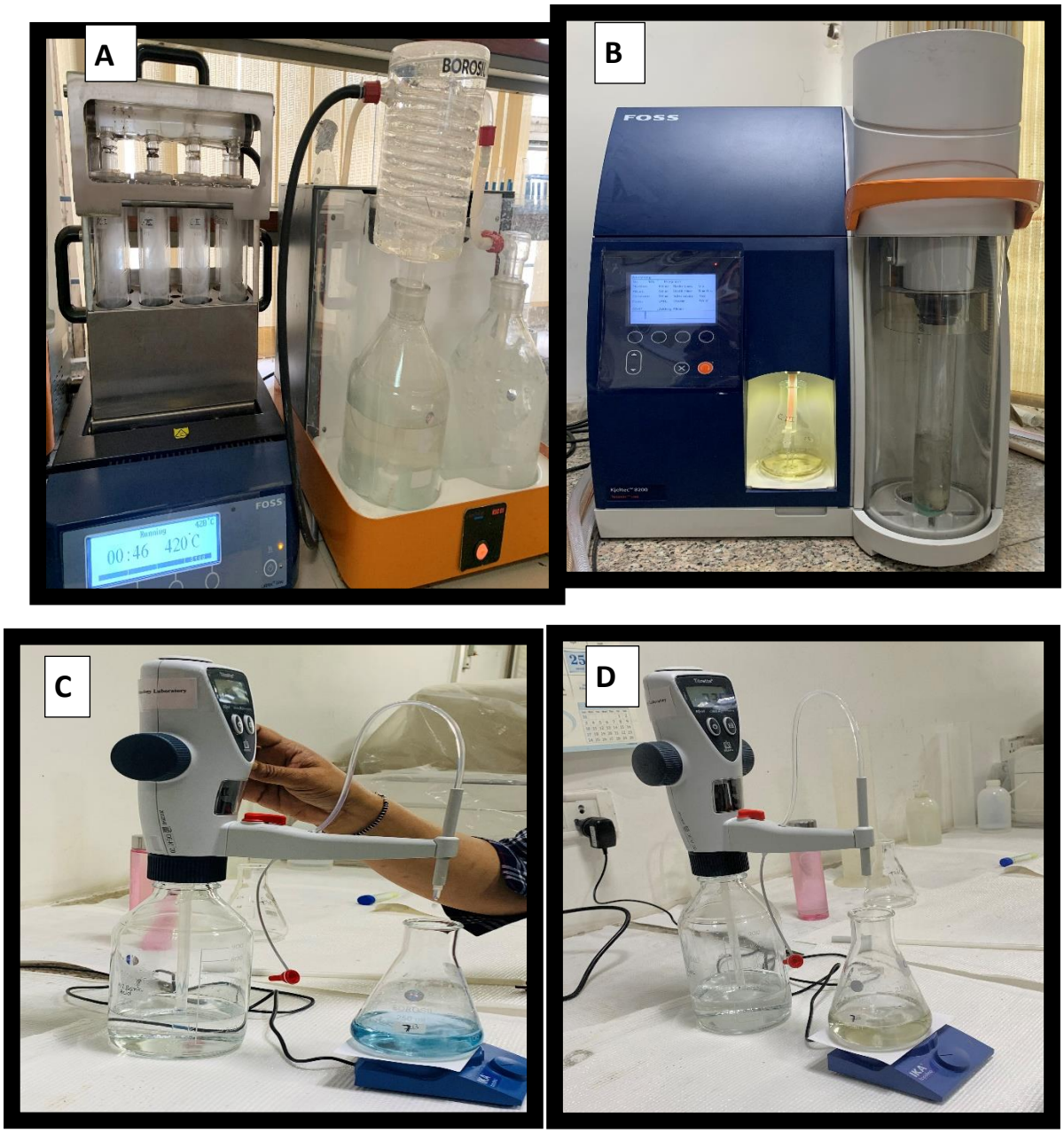


Figure 5- (A) FOSS kjeldahl digester unit (B) Protein Distillation Unit (FOSS KJELTEC 8200)
(C) Titration of Distillate Solution (D) Titration showing protein colorless endpoint

3.10 Statistical Analysis

The results were interpreted using SPSS 21 statistical programme (IBM SPSS Statistics 21) by one way ANOVA with NaCl treatment, microbial inoculation and interactions among them as a source of variation. Comparison of the means were determined by post hoc Duncan's test ($p < 0.05$).

CHAPTER 4 RESULTS

4.1. Shoot and Root Length: As the levels of salinity increased, there was a gradual increase in the shoot and root length in microbial inoculated and uninoculated fenugreek plants. However, inoculation of microbes has notable rise in length of shoot and the root as compared to uninoculated plants at all salinity levels. PGPB inoculated plants showed better results than *P. indica* in terms of number of shoot and root length. The shoot length in PGPB inoculated plants showed an increase by 22.96% and 56.54% at 70 mM and 150 mM NaCl concentrations, respectively. The root length was increased by 7.57% and 29.63% at 70 mM and 150 mM NaCl concentrations, respectively as compared to uninoculated plants.

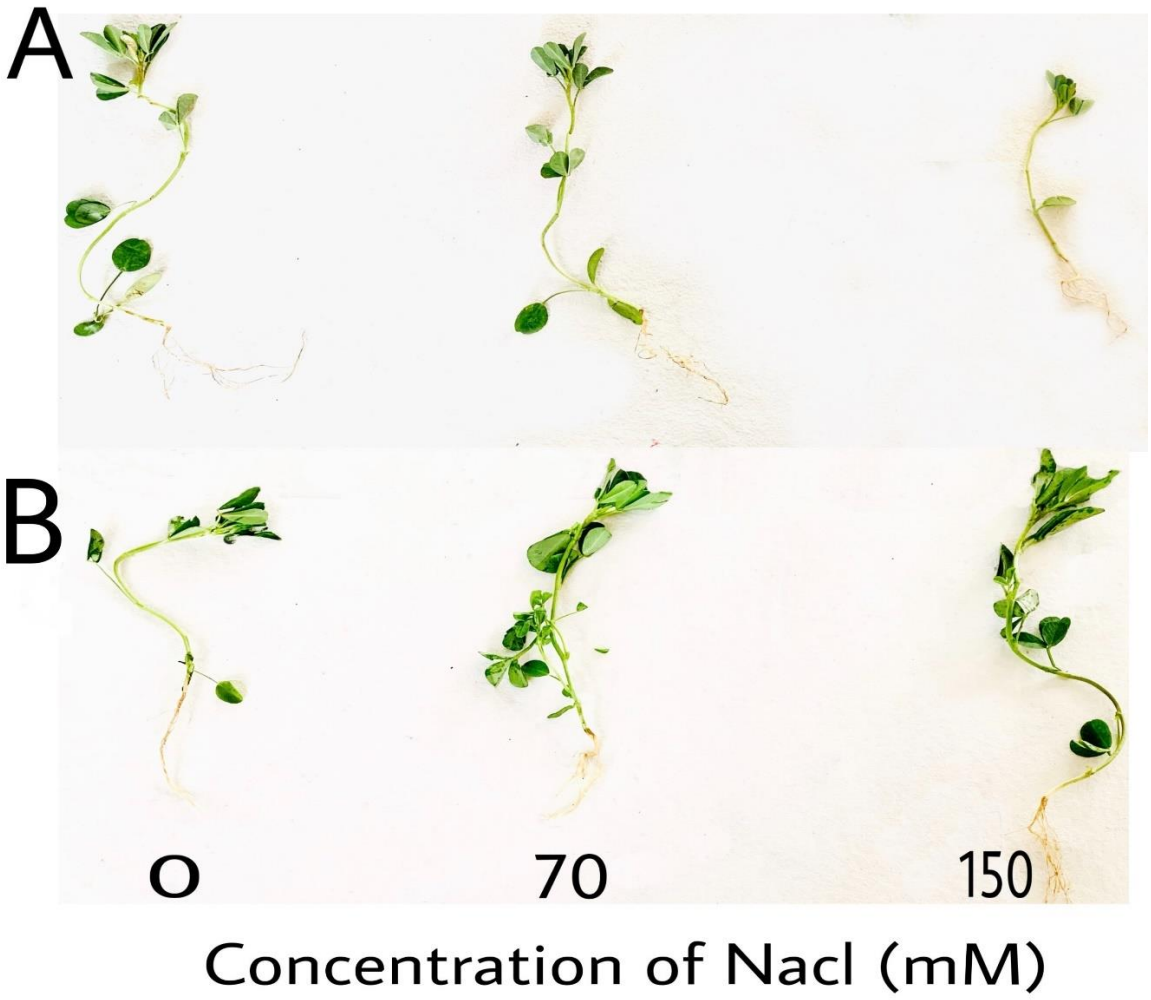
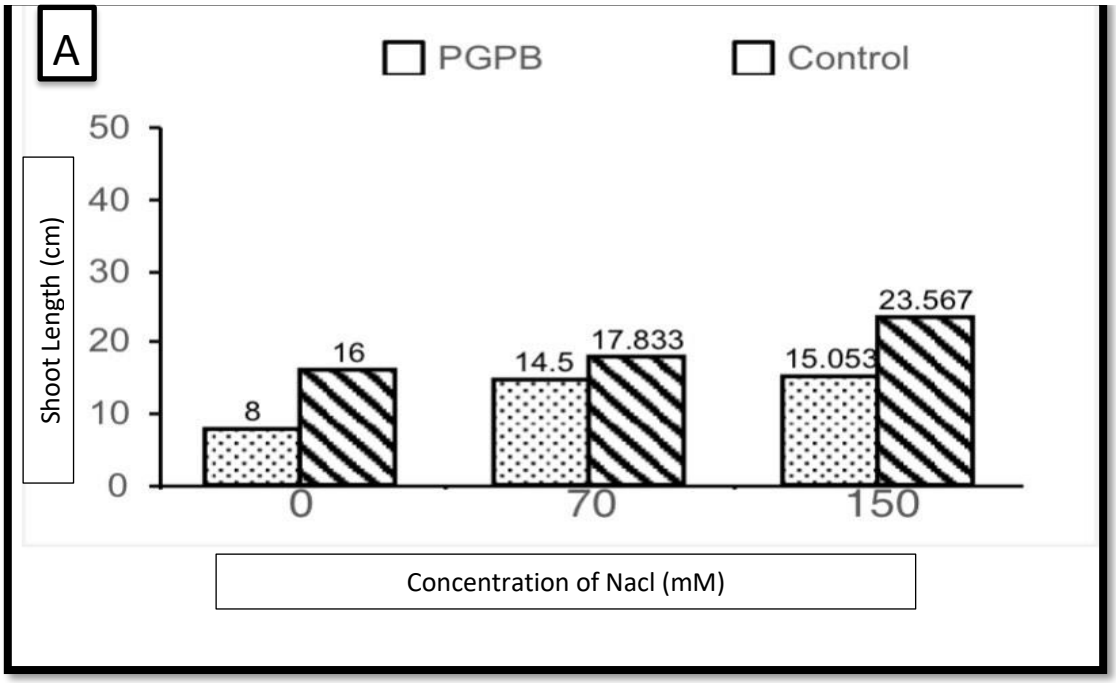


Figure 6- . Effect of Different Concentration of NaCl on Shoot and Root Growth in (A) uninoculated (B) Inoculated with PGPB in Trigonella plants



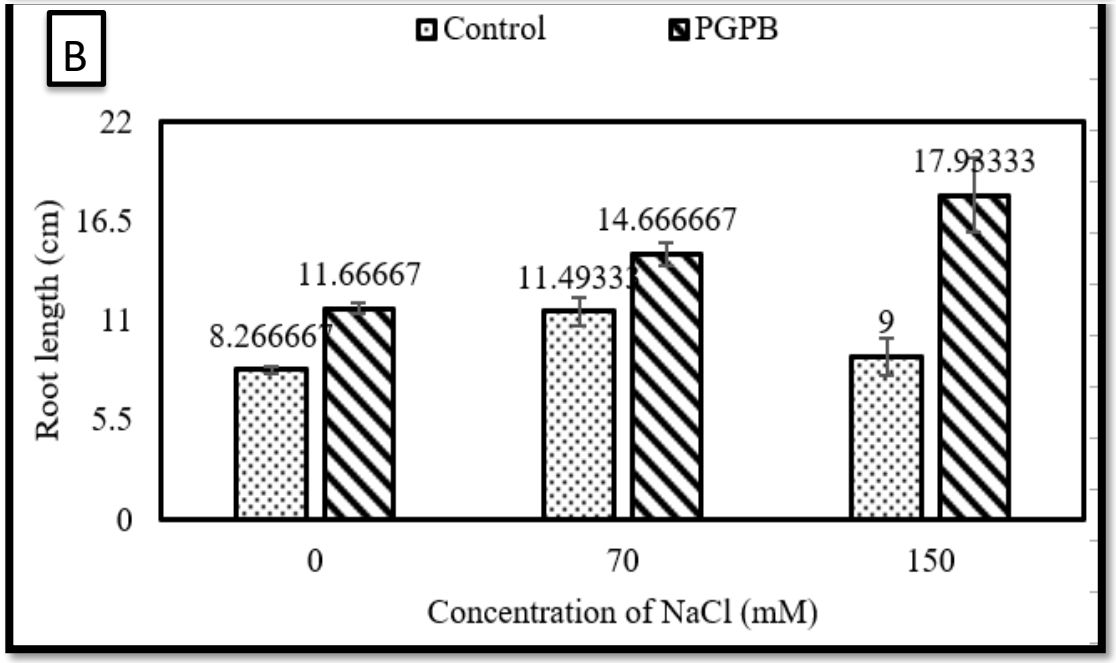


Figure 7- **Effects of different concentration of NaCl on (A) shoot length (B) root length of PGPB Inoculated and uninoculated T. foenum-graecum plants.**

4.2. Biomass: Remarkable positive results were shown by PGPB in elevating the shoot and root dry weight as compared to uninoculated plants. The results showed that PGPB inoculated plants have good impact in elevating the shoot and root dry weight. PGPB showed increase by 55.55% and 209% at 150 mM and 70 mM, respectively as compared to uninoculated plants.

Root dry weight was increased remarkably in PGPB by 110.86% and 207.01% at 150 and 70 mM NaCl concentrations, respectively. Microbial inoculation contributes in the increase of shoot and root dry biomass.

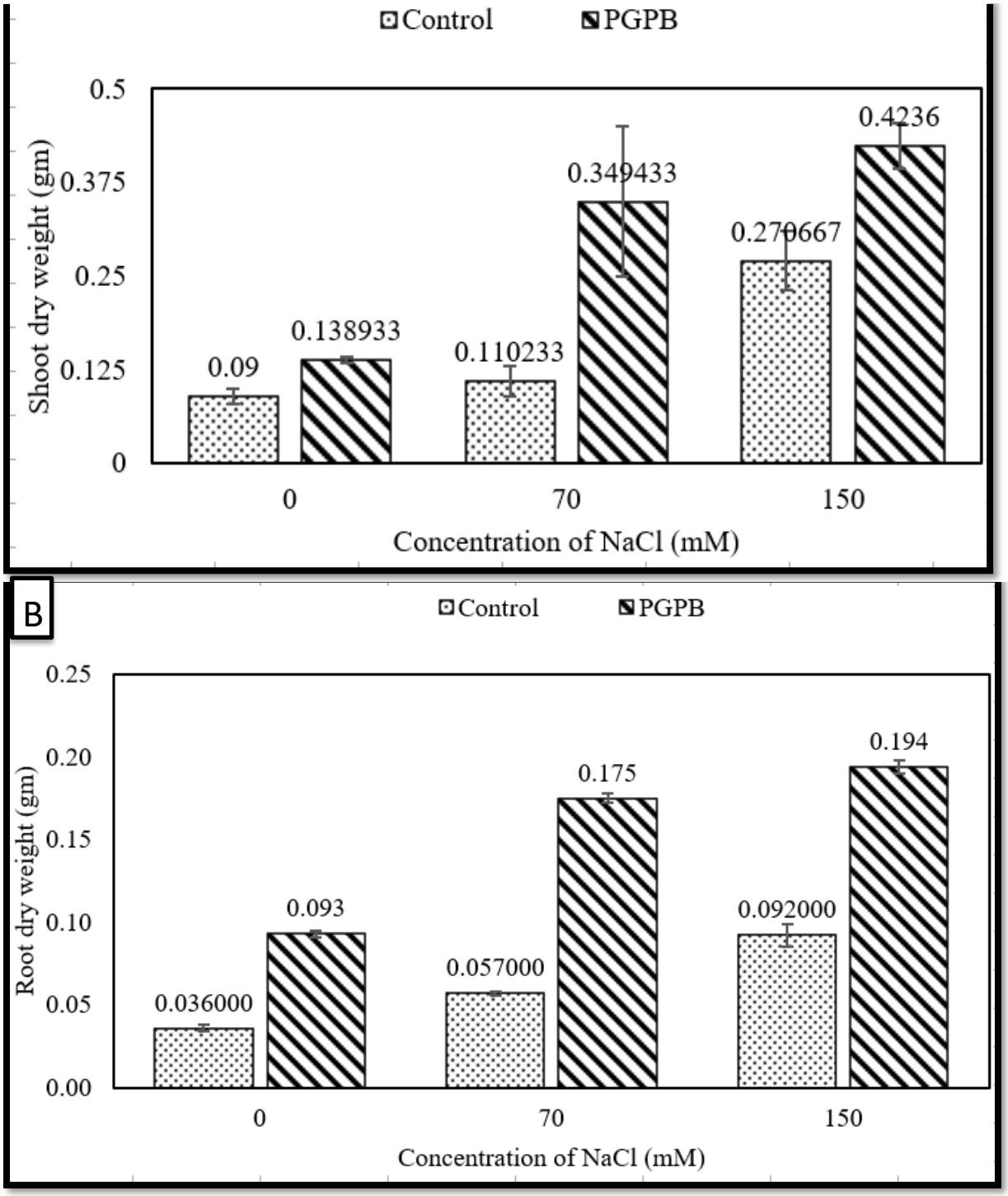


Figure 8- Influence of different concentrations of NaCl on (A) shoot dry weight (B) root dry weight of microbial inoculated and uninoculated T. foenum graecum plants

4.3. Number of Leaves and Leaf Area: Under salinity stress there was a significant difference in the number of leaves and leaf area between microbial inoculated and uninoculated plants. Microbial inoculated plants showed significant rise in number of leaves and leaf area when contrasted to uninoculated plants. PGPB inoculated plants best results in terms of number of leaves and leaf area. The number of leaves in PGPB inoculated plants showed an increase by 78.32% and 37.22% at 70 mM and 150 mM NaCl concentrations, respectively. PGPB showed increase in leaf area by 14.78% and 20.63% at 70 mM and 150 mM NaCl concentration, respectively.

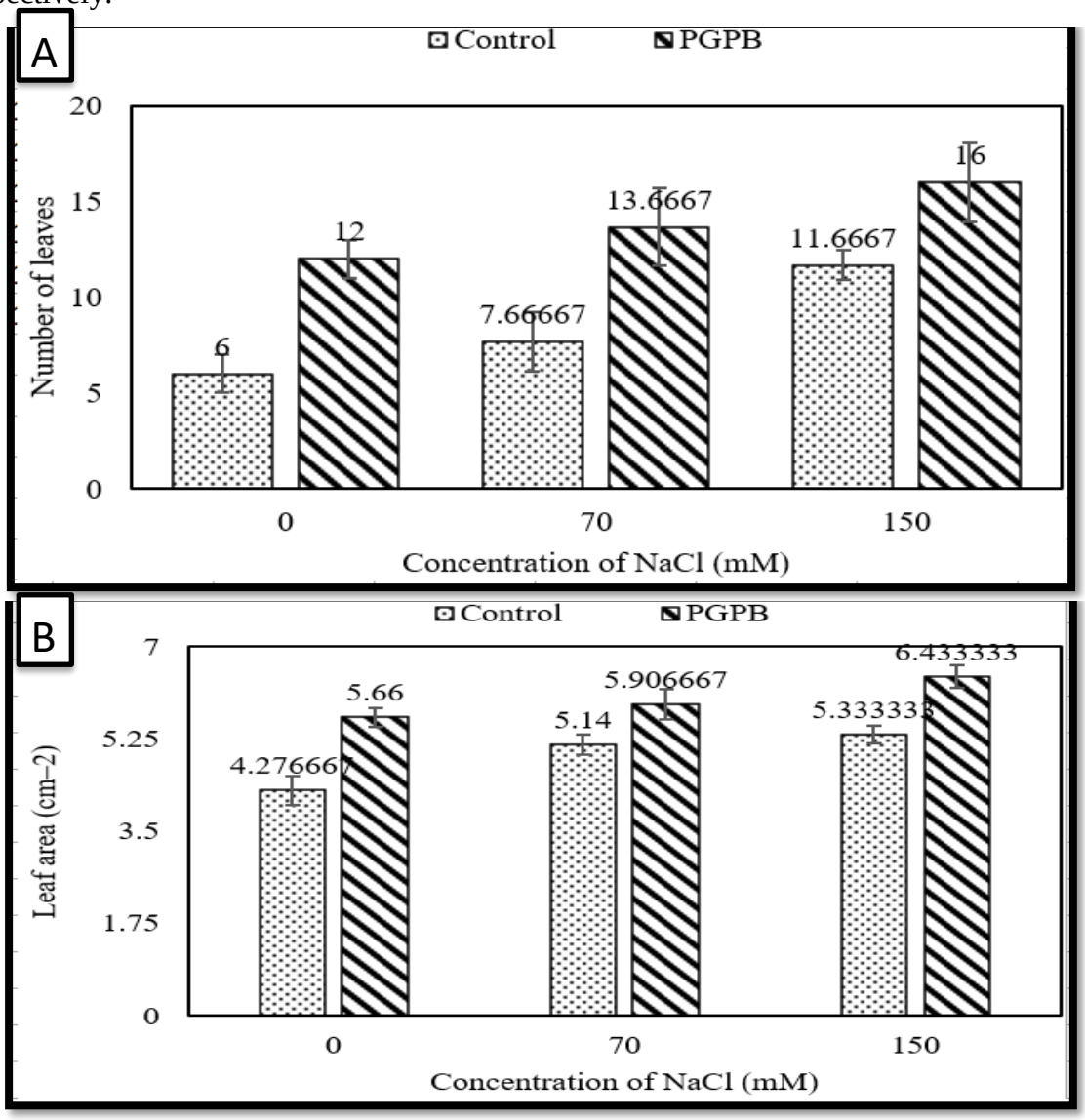
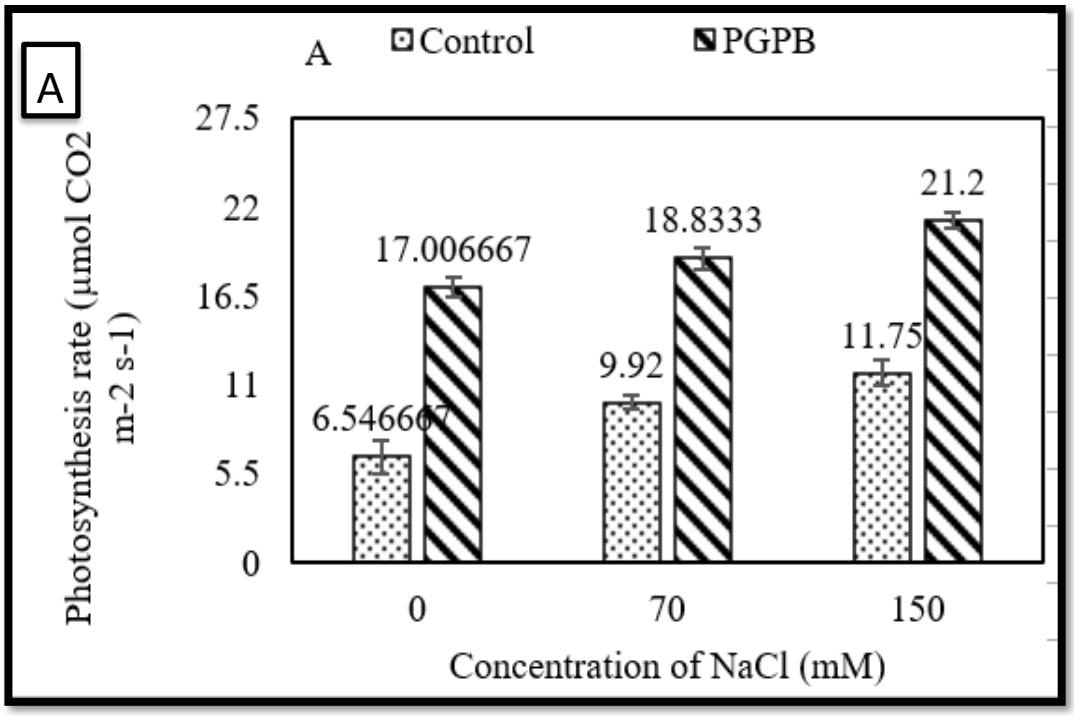


Figure 9- **Effects of different concentration of NaCl (A) number of leaves (B) leaf area of PGPB inoculated and uninoculated T. foenum-graecum plants**.

3.4. Photosynthesis Rate, Stomatal Conductance, Transpiration and Internal CO_2: Under salinity condition there was a significant difference in photosynthesis rate, stomatal conductance, transpiration and internal CO_2 between microbial inoculated and uninoculated plants. Microbial inoculated plants showed increased photosynthesis rate, stomatal conductance, transpiration and internal CO_2 level. In PGPB inoculated plants the photosynthetic rate was significantly increased by 89.81% and 80.42% at 70 mM and 150 mM NaCl concentrations, respectively. In PGPB inoculated plants the stomatal conductance was significantly increased by 107.6% and 150% at 70 mM and 150 mM NaCl concentrations, respectively as contrasted to non treated plants, In PGPB inoculated plants the transpiration rate was significantly increased by 81.46% and 363.9% at 70 mM and 150 mM NaCl concentrations, respectively as compared to uninoculated plants internal CO_2 was significantly increased by 25.79% and 104.38% at 70 mM and 150 mM NaCl concentrations.



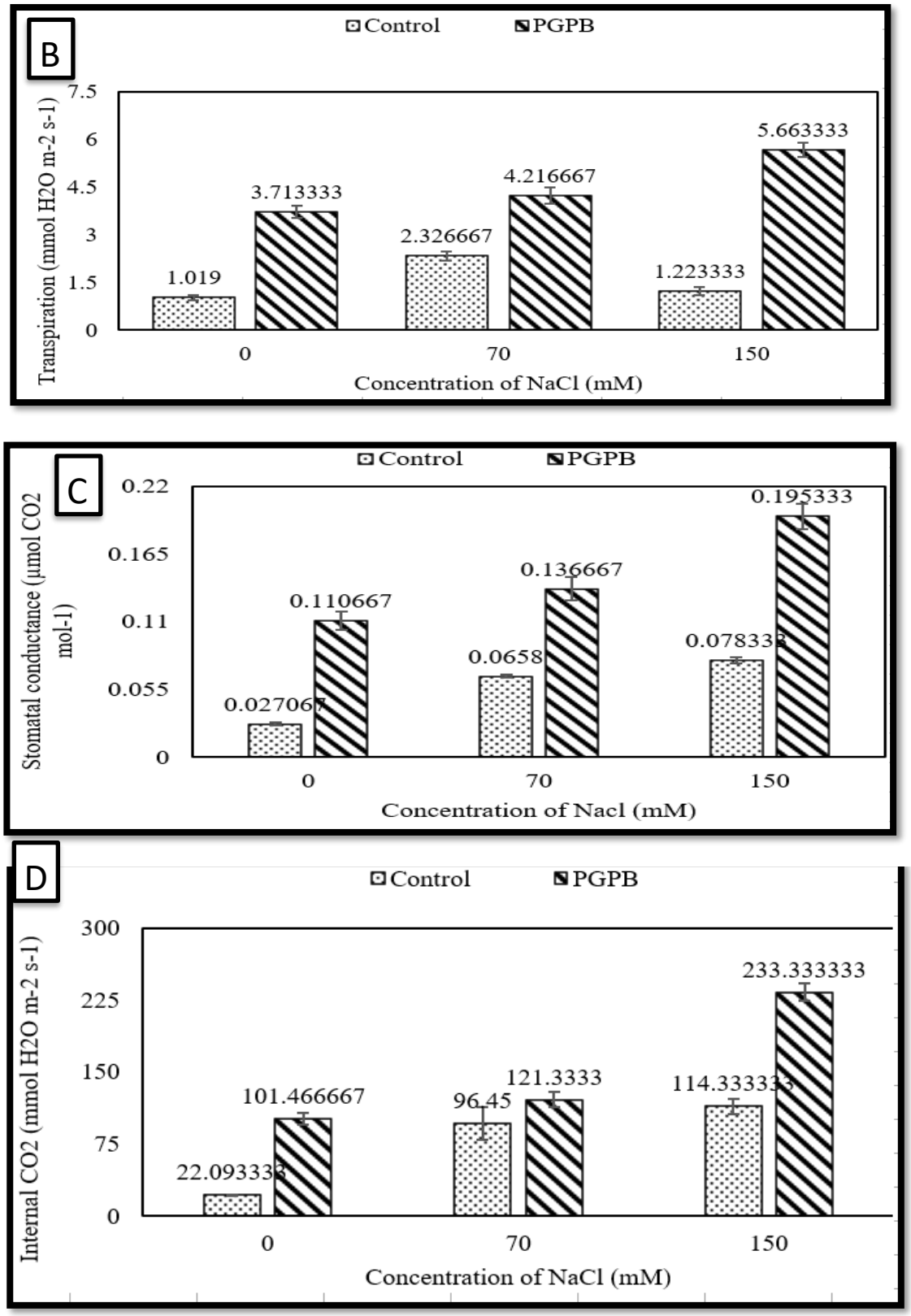
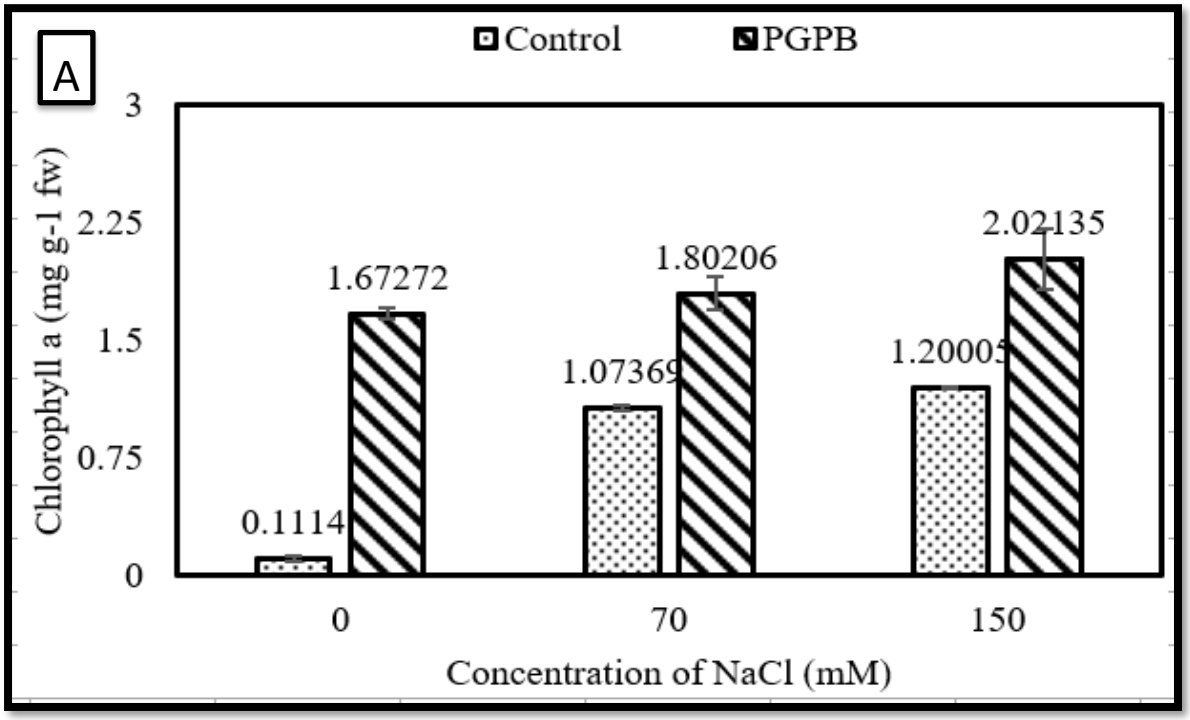
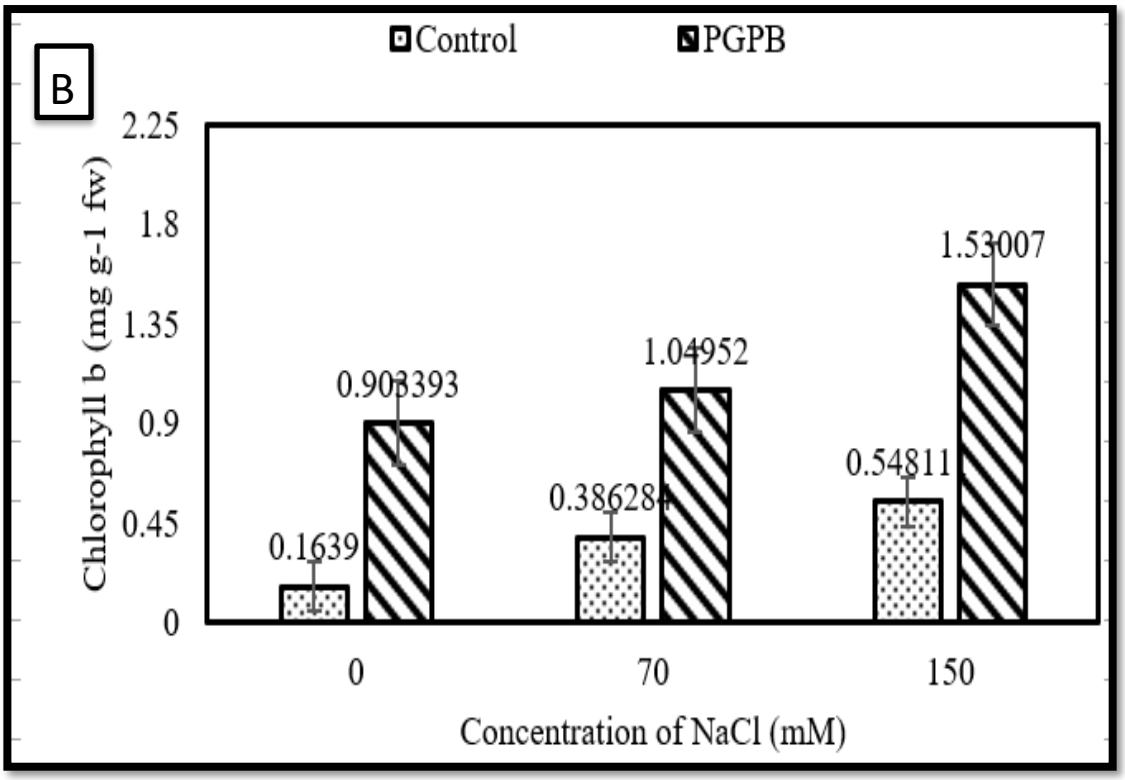
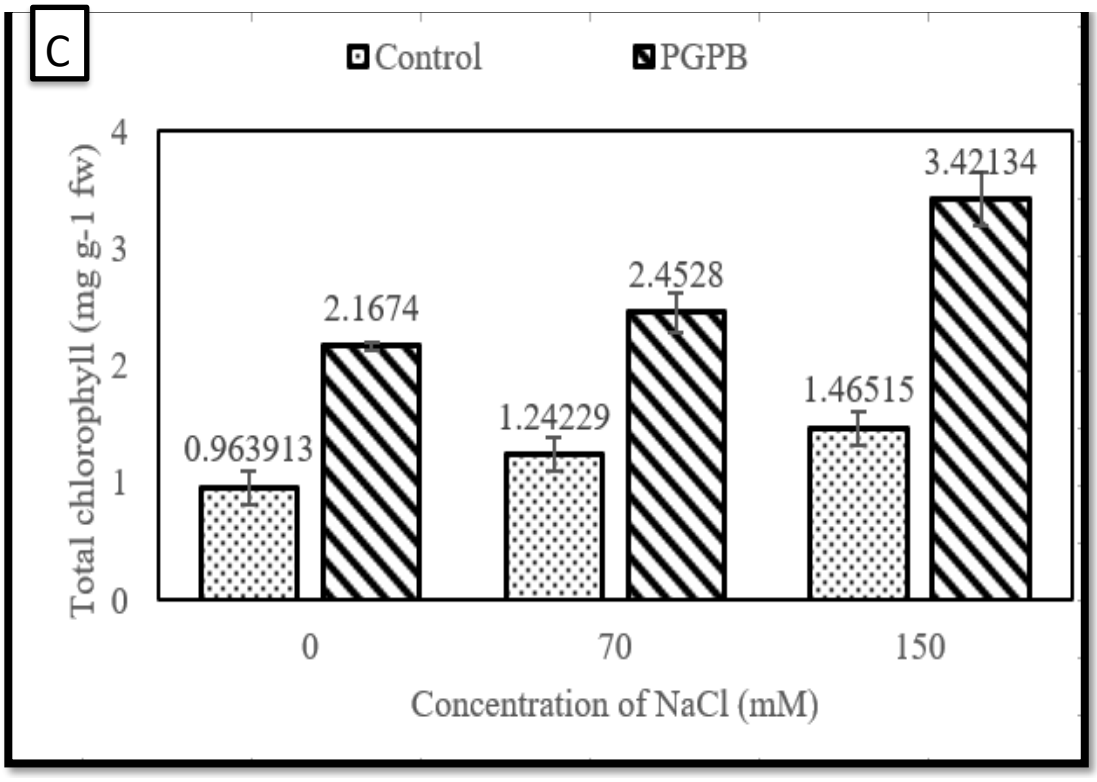


Figure 10- Effects of different concentration of NaCl on (A) photosynthesis (B) stomatal conductance (C) transpiration (D) internal CO 2 of PGPB inoculated and uninoculated T. foenum-graecum plants.

4.5. Photosynthetic Pigments: Under salinity stress there was a significant difference in photosynthetic pigments content between microbial inoculated and uninoculated plants. Microbial inoculated plants showed increased content of photosynthetic pigments as compared to uninoculated plants. PGPB inoculated plants showed maximum results in content of photosynthetic pigments. In PGPB inoculated plants carotenoid content was significantly increased by 86.36% and 77.35% at 70 mM and 150 mM NaCl concentrations, respectively as contrasted to plants with no inoculation, In PGPB inoculated plants the chlorophyll a content was significantly increased by 68.22% and 68.33% at 70 mM and 150 mM NaCl concentrations, respectively as compared to uninoculated plants, In PGPB inoculated plants the chlorophyll b content was significantly increased by 173.68% and 183.3% at 70 mM and 150 mM NaCl concentration, respectively , the total chlorophyll content was significantly increased by 80.64% and 134.25% at 70 mM and 150 mM NaCl concentrations, respectively.




B
Control
PGPB
Chlorophyll b (mg g-1 fw)
2.25
1.8
1.35
0.9
0.45
0
0.1639
0.903393
0.386284
1.04952
0.54811
1.53007
0
70
150
Concentration of NaCl (mM)


C
Control
PGPB
Total chlorophyll (mg g-1 fw)
4
3
2
1
0
0.963913
2.1674
1.24229
2.4528
1.46515
3.42134
0
70
150
Concentration of NaCl (mM)

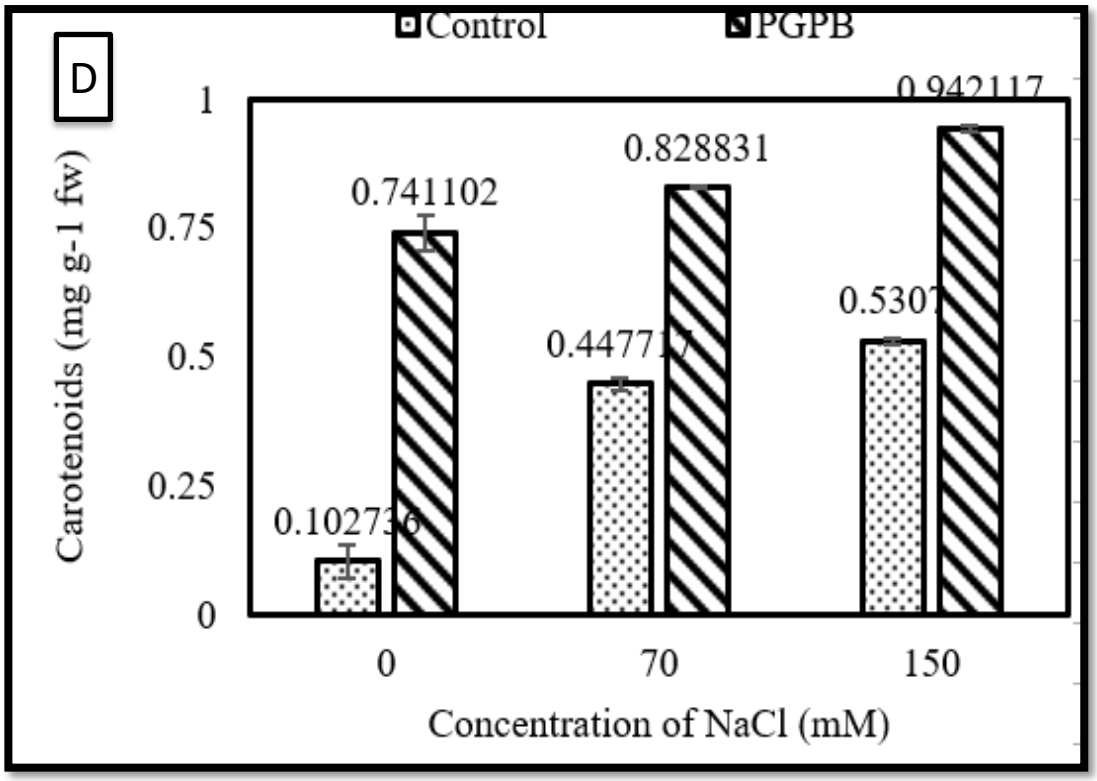


Figure 11- Effects of different concentration of NaCl on (A) carotenoids (B) chlorophyll a (C) chlorophyll b (D) total chlorophyll content of PGPB inoculated and uninoculated T. foenum-graecum plants.

4.6. Nitrogen and Protein: During salinity stress there was a significant difference in nitrogen and protein content between microbial inoculated and uninoculated plants. Microbial inoculated plants showed increased content of nitrogen and protein as compared to uninoculated plants. PGPB inoculated plants showed significant results in nitrogen and protein content. In PGPB inoculated plants nitrogen and protein content was significantly enhanced by 53.26% and 40.78% at 70 mM and 150 mM NaCl concentrations, respectively.

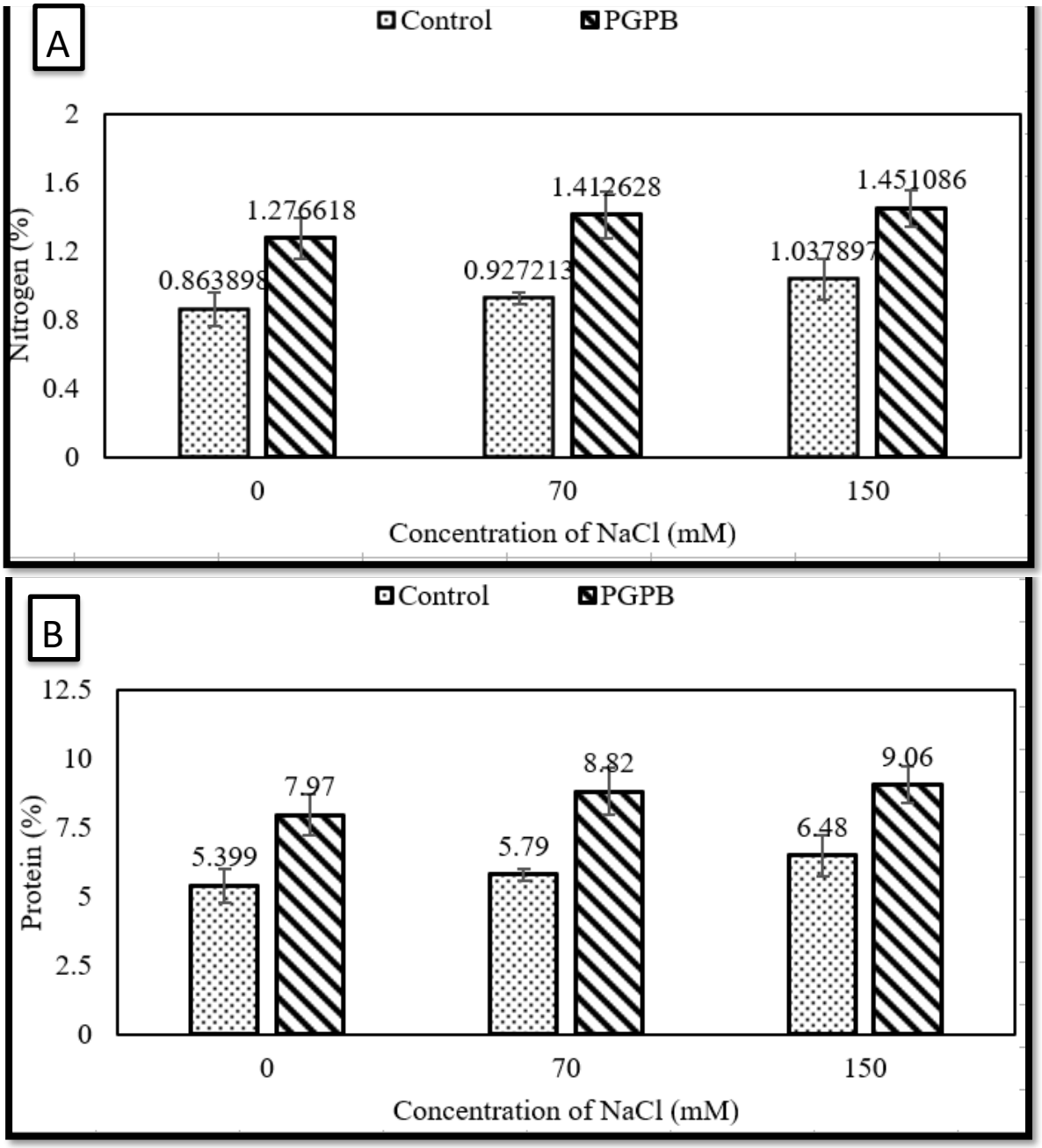


Figure 12- Effect of different concentrations of NaCl on (A) nitrogen and (B) protein content of PGPB and uninoculated T. foenum-graecum plants.

CHAPTER 5 DISCUSSIONS

Inoculation with PGPB had elevated the morphological responses like increased shoot and root length, under high salinity conditions, which was unlike in the uninoculated plants. PGPB showed best results in increasing root and shoot length. Due to enhanced intake of surplus amount of nutrients like nitrogen and many other essential nutrients, when inoculated with beneficial micro-organisms the shoot and root length was increased. (Gupta and Pandey,2019) have also showed increase in shoot and root length in french beans seedlings under salinity stress when inoculated with the strains of PGPB which are ACC02 and ACC06, respectively.

Microbial inoculation showed a presented a remarkable result in elevating the biomass as contrasted to non-inoculated plants. The results depicted that PGPB inoculated plants had significant impact elevating the biomass of plant. Elevation in both the shoot and the root biomass in the microbial inoculation were because of the increase the protein content, nitrogen and photosynthetic rate. Uninoculated plants showed poor results. Increase in the shoot and the root's dry mass were reported by (Hajiboland et al., 2015) in *Aeluropus littoralis* when inoculated with fungi *Claroideoglomus etunicatum* under salinity stress.

A major difference was seen in the number of the leaves and the leaf area between microbial inoculated and uninoculated plants under salinity stress. Microbial inoculated plants showed significant rise in the number of their leaves and leaf area when put in contrast to uninoculated plants, PGPB inoculated plants depicted good results as far as the number of leaves and leaf area were concerned. The increase in number of leaves was might due to the division of cells causing change in leaf number. Leaf area was found to be highin the PGPB inoculation as compared to uninoculated under extreme salinity stress. Leaf area is one of the most important factors which directly co-relates with the photosynthetic active area. Elevation in leaf area was caused because of intake of various inorganic and organic nutrients, water uptake (Khalloufi, et al)have showed a rise in the amount of leaves (leaf count) and leaf area under stress of saline when inoculated with fungi *Rhizophagus irregularis* in *Solanum lycopersicum* L. plants.

Microbial inoculation was very beneficial as it improved various physiological parameters like photosynthesis rate, stomatal conductance, transpiration and internal CO_2 even under high salinity stress. PGPB inoculated plants showed remarkable results in elevating photosynthetic rate, stomatal conductance, transpiration and also internal CO_2. Photosynthetic rate was

increased in the microbial inoculation even under the high salinity stress because of high leaf area which directly co-relates with the photosynthetic efficiency of plants. Stomatal conductance is a measure of the degree of the stomatal opening and acts as an indicator of plant water status, increase in the stomatal conductance might be due to plant-water relations. Transpiration, on the other hand, was increased due to the increased utilization of water during photosynthesis which created transpiration pull. Increased internal CO_2 content enhanced the photosynthesis rate, plant growth and development. Increased internal CO_2 wasdue to increased stomatal conductance. The photosynthetic efficiency was increased in *Ocimum basilicum* L. when inoculated with arbuscular mycorrhizal fungi (*Glomus deserticola*) under elevated salinity stress (Elhindi et al., 2017).

A major difference was seen in photosynthetic pigments content among the microbial inoculated and the uninoculated plants. Microbial inoculated plants depicted rise in content of photosynthetic pigments as contrasted to the uninoculated ones. PGPB inoculated plants showed remarkable results in terms of content of photosynthetic pigments viz. chlorophyll a, chlorophyll b, total chlorophyll and carotenoid.Increased content of photosynthetic pigments might be because of increased uptake of the nutrients. Under salinity stress, seeds inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens* were the reason for the significant rise in the photosynthetic pigments of radish plants (Mohamed and gomaa, 2012)

During salinity stress there was a notable difference in nitrogen and protein content between microbial inoculated and uninoculated plants. Microbial inoculated plants showed increased content of nitrogen and protein as compared to uninoculated plants. PGPB showed remarkable results in elevating nitrogen and protein. Nitrogen is an important part of chlorophyll, through which the plant uses the sunlight and produces sugars and oxygen. Also, nitrogen is the building blocks of the amino acids, rise in the amount of nitrogen co-related with the rise of protein quantity in the plants. Protein act as osmolyte maintain the osmotic balance during stress condition. Increased salinity tolerance in fenugreek plant might be due to enhanced production of protein. Nitrogen content was also increased in *Acacia saligna* (Labill.) under high salinity stress when inoculated with arbuscular mycorrhizal fungi

CHAPTER 6 CONCLUSION

PGPB inoculated fenugreek plants showed enhanced morphological attributes (shoot and root's length, their dry mass and leaf count and leaf area) and physiological responses (photosynthesis, stomatal conductance, transpiration, internal CO_2) during salinity stress as contrasted to uninoculated plants. The outcome was presented in this investigation clearly which depicted that PGPB improved salt stress tolerance potential of fenugreek plants, by enhanced accumulation of carotenoids, chlorophyll a, chlorophyll b, total chlorophyll, nitrogen and protein content in plants during salinity stress. The improved biochemical responses and physiological responses in PGPB inoculated plants under the stress of salinity, also indicate that plant-microbe interaction could mitigate salinity stress in fenugreek plant.

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